

Claims:

1. A solid phase based nucleic acid assay that can distinguish alleles which comprise a single nucleotide polymorphism in a sample containing a target nucleic acid molecule comprising
 - a) immobilizing the target nucleic acid molecule to a solid support using
 - i) one or more capture oligonucleotides that are immobilized to the solid support, that hybridize to the target molecule and that have terminal nucleotides blocked and/or unphosphorylated terminal nucleotides and
 - ii) a discrimination oligonucleotide that are immobilized to the solid support, that hybridize to the target molecule wherein a nucleotide at a terminus of the discrimination oligonucleotide is complementary to the single nucleotide polymorphism position and is unblocked and/or phosphorylated;
 - b) performing a reaction on any unblocked and/or phosphorylated terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for unblocked and/or phosphorylated terminal nucleotide perfectly hybridized to a complementary nucleotide; and
 - c) determining if any reaction occurred, wherein detecting a reaction of unblocked and/or phosphorylated terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination oligonucleotide hybridized perfectly to the target nucleic acid molecule at the single nucleotide polymorphism position.
2. A solid phase based nucleic acid assay of claim 1 comprising:
 - a) contacting under hybridizing conditions a target nucleotide molecule, one or more target capture extenders, a discrimination extender and a solid support that comprises one or more target capture probes and a capture probe immobilized at the 3' terminus to the solid support directly or with spacers, wherein:
 - i) the discrimination extender comprises a sequence that is complementary to a sequence of the capture probe sufficient to immobilize the discrimination extender to the solid support, and a sequence that is complementary to a sequence of the target nucleic acid molecule including a sequence complementary to an allele that comprises a single nucleotide polymorphism in

which the nucleotide complementary to the single nucleotide polymorphism position is at an unblocked 3' terminal nucleotide of the discrimination extender,

ii) the capture probe comprises a sequence that is complementary to a sequence of the discrimination extender sufficient to immobilize the discrimination extender to the solid support,

iii) the one or more target capture probes comprise a sequence that is complementary to a sequence of one or more target capture extenders each sufficient to immobilize the target capture extender to the solid support, each target capture extender further comprising a sequence that is complementary to a sequence of the target nucleic acid molecule each sufficient to hybridize the target nucleic acid molecule to the target capture extender, each target capture extender having a blocked 3' terminal nucleotide; and

iv) the one or more target capture extenders hybridize to the one or more target capture probes on the solid support and to the target nucleic acid molecule, and the discrimination extender hybridizes to the capture probe on the solid support and to the target nucleic acid molecule;

b) performing a reaction on any unblocked 3' terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide; and

c) determining if any reaction occurred, wherein detecting a reaction of unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination extender hybridized perfectly to the target nucleic acid molecule at the 3' terminal nucleotide and that the allele present in the target nucleic acid molecule is the allele that has the single nucleotide polymorphism complementary to the 3' terminus of the discrimination extender.

3. A solid phase based nucleic acid assay of claim 1 comprising:

a) contacting under hybridizing conditions a target nucleotide molecule, one or more target capture extenders, a discrimination extender and a solid support that comprises one or more target capture probes and a capture probe immobilized at the 5' terminus to the solid support directly or with spacers, wherein:

i) the discrimination extender comprises a sequence that is complementary to a sequence of the capture probe sufficient to immobilize the discrimination extender to the solid support, and a sequence that is complementary to a sequence of the target nucleic acid molecule including a sequence complementary to an allele that comprises a single nucleotide polymorphism in which the nucleotide complementary to the single nucleotide polymorphism position is at a phosphorylated 5' terminal nucleotide of the discrimination extender,

ii) the capture probe comprises a sequence that is complementary to a sequence of the discrimination extender sufficient to immobilize the discrimination extender to the solid support,

iii) the one or more target capture probes comprise a sequence that is complementary to a sequence of one or more target capture extenders each sufficient to immobilize the target capture extender to the solid support, each target capture extender further comprising a sequence that is complementary to a sequence of the target nucleic acid molecule each sufficient to hybridize the target nucleic acid molecule to the target capture extender, each target capture extender having an unphosphorylated 5' terminal nucleotide; and

iv) the one or more target capture extenders hybridize to the one or more target capture probes on the solid support and to the target nucleic acid molecule, and the discrimination extender hybridizes to the capture probe one the solid support and to the target nucleic acid molecule;

b) performing a reaction on any phosphorylated 5' terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide; and

c) determining if any reaction occurred, wherein detecting a reaction of unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination extender hybridized perfectly to the target nucleic acid molecule at the phosphorylated 5' nucleotide and that the allele present in the target nucleic acid molecule is the allele that has the single

nucleotide polymorphism complementary to the 5' terminus of the discrimination extender.

4. A solid phase based nucleic acid assay of claim 1 comprising:

a) contacting under hybridizing conditions a target nucleotide molecule, one or more target capture extenders, a discrimination extender, and a solid support that comprises one or more target capture probes and a capture probe immobilized at the 3' terminus to the solid support directly or with spacers and one or more target capture probes immobilized at the 5' terminus to the solid support directly or with spacers, wherein:

i) the discrimination extender comprises a sequence that is complementary to a sequence of the capture probe sufficient to immobilize the discrimination extender to the solid support, and a sequence that is complementary to a sequence of the target nucleic acid molecule including a sequence complementary to an allele that comprises a single nucleotide polymorphism in which the nucleotide complementary to the single nucleotide polymorphism position is at an unblocked 3' terminal nucleotide of the discrimination extender,

ii) the capture probe comprises a sequence that is complementary to a sequence of the discrimination extender sufficient to immobilize the discrimination extender to the solid support,

iii) one or more target capture probes comprise a sequence that is complementary to a sequence of one or more target capture extenders each sufficient to immobilize the target capture extender to the solid support, each target capture extender further comprising a sequence that is complementary to a sequence of the target nucleic acid molecule each sufficient to hybridize the target nucleic acid molecule to the target capture extender, each target capture extender and each target capture probe having a blocked 3' terminal nucleotide; and

iv) the one or more target capture extenders hybridize to the one or more target capture probes on the solid support and to the target nucleic acid molecule, and the discrimination extender hybridizes to the capture probe on the solid support and to the target nucleic acid molecule;

c) performing a reaction on any unblocked 3' terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for

unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide; and

d) determining if any reaction occurred, wherein detecting a reaction of unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination extender hybridized perfectly to the target nucleic acid molecule at the 3' terminal nucleotide and that the allele present in the target nucleic acid molecule is the allele that has the single nucleotide polymorphism complementary to the 3' terminus of the discrimination extender.

5. A solid phase based nucleic acid assay of claim 1 comprising:

a) contacting under hybridizing conditions a target nucleic acid molecule and a solid support that comprises one or more target capture probes and a discrimination probe linked to the solid support at the 5' terminus directly or with spacers, wherein:

i) the discrimination probe comprises a sequence that is complementary to a sequence of the target nucleic acid molecule including a sequence complementary to an allele that comprises a single nucleotide polymorphism in which the nucleotide complementary to the single nucleotide polymorphism position is at an unblocked 3' terminal nucleotide of the discrimination probe,

ii) the one or more target capture probes comprise a sequence that is complementary to one or more sequences of the target nucleic acid molecule each sufficient to hybridize the target nucleic acid molecule to the capture probe, each capture probe having a blocked 3' terminal nucleotide;

b) performing a reaction on any unblocked 3' terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide; and

d) determining if any reaction occurred, wherein detecting a reaction of unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination probe hybridized perfectly to the target nucleic acid molecule at the 3' terminal nucleotide and that the allele present in

the target nucleic acid molecule is the allele that has the single nucleotide polymorphism complementary to the 3' terminus of the discrimination probe.

6. A solid phase based nucleic acid assay of claim 1 comprising:

a) contacting under hybridizing conditions a target nucleic acid molecule and a solid support that comprises one or more target capture probes and a discrimination probe linked to the solid support at the 3' terminus directly or with spacers, wherein:

i) the discrimination probe comprises a sequence that is complementary to a sequence of the target nucleic acid molecule including a sequence complementary to an allele that comprises a single nucleotide polymorphism in which the nucleotide complementary to the single nucleotide polymorphism position is at a phosphorylated 5' terminal nucleotide of the discrimination probe,

ii) the one or more target capture probes comprise a sequence that is complementary to one or more sequences of the target nucleic acid molecule each sufficient to hybridize the target nucleic acid molecule to the capture probe, each capture probe having an unphosphorylated 5' terminal nucleotide;

b) performing a reaction on any phosphorylated 5' terminal nucleotide hybridized to a complementary nucleotide, the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide; and

d) determining if any reaction occurred, wherein detecting a reaction of phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide indicates the discrimination probe hybridized perfectly to the target nucleic acid molecule at the 5' terminal nucleotide and that the allele present in the target nucleic acid molecule is the allele that has the single nucleotide polymorphism complementary to the 5' terminus of the discrimination probe.

7. The assay of any of claims 1-5 comprising a reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or a reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide, wherein the reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary

nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide is an enzymatic ligation reaction of a labeling nucleotide or oligonucleotide to a discrimination probe or a discrimination extender having an unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide.

8. The assay of any of claims 1-5 comprising a reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide, wherein the reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide is a polymerase catalyzed primer extension reaction comprising incorporation of labeled nucleotides.

9. The assay of any of claims 1, 2, 4 or 5 comprising a reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide, wherein the reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide is a polymerase catalyzed primer extension reaction comprising incorporation of labeled nucleotides.

10. The assay of any of claims 1, 2, 4 or 5 comprising a reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide, wherein the reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide is a polymerase catalyzed single base extension reaction comprising incorporation of labeled nucleotides.

11. The assay of any of claims 1-6 comprising a reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide, wherein the reaction specific for unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide is a chemical ligation reaction

of a labeling entity to a discrimination probe or a discrimination extender having an unblocked 3' terminal nucleotide perfectly hybridized to a complementary nucleotide or the reaction being specific for phosphorylated 5' terminal nucleotide perfectly hybridized to a complementary nucleotide.

12. The assay of claim 11 wherein the labeled entity is a labeled oligonucleotide.

13. The assay of any of claims 7-12 wherein labeled nucleotides are labeled with biotin, a fluorophore, a nanoparticle, or an enzymes.

14. The assay of any of claims 1-13 wherein the solid support is a bead, a planar surface, a metallic particle, or a gel matrix.

15. The assay of any of claims 1-14 wherein determination of whether a reaction occurred use optical, electrical, mechanical, or magnetic methods.

16. The assay of any of claims 1-15 wherein the assay is a multiplex assay to identify the presence of multiple genes having alleles that comprise a single nucleotide polymorphism, comprising an assay that comprises multiple discrimination probes that are each specific for a single gene that has alleles that comprise a single nucleotide polymorphism and discrimination probes and target capture probes are spatially separated on specific spots on a solid support, wherein the assay comprises multiple discrimination probes that are each specific for a single gene that has alleles that comprise a single nucleotide polymorphism and discrimination probes and target capture probes are spatially separated on specific spots on a solid support, or the assay comprises multiple discrimination extenders that are each specific for a single gene that has alleles that comprise a single nucleotide polymorphism and capture probes and target capture probes are spatially separated on specific spots on a solid support that are immobilized to the solid support in a spatial separated n or discrimination extenders.

17. The assay of any of claims 2-4 wherein the discrimination extender is hybridized to the capture probe immobilized to the solid support prior to contacting with the target nucleic acid molecule.

18. The assay of claim 17 wherein the target capture extenders are mixed with the target nucleic acid molecule prior to contacting with the target capture probes immobilized on the solid support.

19. The assay of claim 17 wherein the target capture extenders are mixed with the target capture probes immobilized on the solid support prior to contacting with the target nucleic acid molecule.

20. A kit comprising a solid support selected from the group consisting of a solid support that comprises:

a) a capture probe and one or more target capture probes linked to the solid support at the 3' terminus directly or with spacers, one or more target capture extenders with blocked 3' termini and sequences complementary to a sequence on a target capture probe and a target nucleic acid molecule, and a discrimination extender with an unblocked 3' terminus that and a sequence complementary to a sequence on a capture probe and a target nucleic acid molecule wherein the nucleotide at the 3' terminus of the discrimination extender is complementary to a single nucleotide polymorphism position of an allele;

b) a capture probe and one or more target capture probes linked to the solid support at the 5' terminus directly or with spacers, one or more target capture extenders with unphosphorylated 5' termini and sequences complementary to a sequence on a target capture probe and a target nucleic acid molecule, and a discrimination extender with an phosphorylated 5' terminus and a sequence complementary to a sequence on a capture probe and a target nucleic acid molecule wherein the nucleotide at the 5' terminus of discrimination extender is complementary to a single nucleotide polymorphism position of an allele;

c) a capture probe linked to the solid support at the 3' termini directly or with spacers, one or more target capture probes with blocked 3' termini and linked to the solid support at the 5' terminus directly or with spacers, one or more target capture extenders with blocked 3' termini and sequences complementary to a sequence on a target capture probe and a target nucleic acid molecule, and a discrimination extender with an unblocked 3' terminus and a sequence complementary to a sequence on a capture probe and a target nucleic acid molecule wherein the nucleotide at the 3' terminus of the discrimination extender is complementary to a single nucleotide polymorphism position of an allele; and

combination thereof.

21. A kit of claim 20 wherein the solid support comprises more than one different capture probe that hybridizes to different discrimination extenders, each different capture probe spatially separated at identifiable locations and different discrimination extenders having termini complementary to a single nucleotide polymorphism position of an allele of different genes.

22. A solid support selected from the group consisting of:

a) a solid support comprising a discrimination probe linked to the solid support at the 5' termini directly or with spacers, one or more target capture probes with blocked 3' termini and linked to the solid support at the 5' terminus directly or with spacers, wherein a sequence of a target probe is complementary to a sequence on a target nucleic acid molecule and a sequence on the discrimination probe is complementary to a sequence on the target nucleic acid molecule wherein the nucleotide at the 3' terminus of the discrimination probe is unblocked and complementary to a single nucleotide polymorphism position of an allele;

b) a solid support comprising a discrimination probe linked to the solid support at the 3' termini directly or with spacers, one or more target capture probes with unphosphorylated 5' termini and linked to the solid support at the 3' terminus directly or with spacers, wherein a sequence of a target probe is complementary to a sequence on a target nucleic acid molecule and a sequence on the discrimination probe is complementary to a sequence on the target nucleic acid molecule wherein the nucleotide at the 5' terminus of the discrimination probe is phosphorylated and complementary to a single nucleotide polymorphism position of an allele; and

combinations thereof.

23. A solid support of claim 22 wherein the solid support comprises more than one different discrimination probe, each different discrimination probe spatially separated at identifiable locations and different discrimination extenders having termini complementary to a single nucleotide polymorphism position of an allele of different genes.